

Certificate of Analysis for NR-51615

Entamoeba invadens, Strain CK (+luc) (axenic)

Catalog No. NR-51615

(Derived from ATCC® 30994™)

Product Description:

Entamoeba invadens (E. invadens), strain CK (+luc) is a transgenic clone derived from strain IP-10 (ATCC[®] 30994™; BEI Resources NR-15226), which was originally isolated in 1952 from a green water snake (Natrix cyclopion) in Florida. Strain CK (+luc) was deposited to BEI Resources as a firefly luciferase-expressing strain. NR-51615 was produced by cultivation of ATCC[®] 30994™ in LYI Entamoeba medium supplemented with 10% heat-inactivated adult bovine serum (HIBS), Diamond's Vitamin solution and 40 μg per mL G418 for 10 days at 25°C in an axenic and microaerophilic atmosphere until peak density was reached.

Lot: 70022612 Manufacturing Date: 22MAR2019

TEST	SPECIFICATIONS	RESULTS
Cellular Morphology¹ 2 days at 25°C in a microaerophilic atmosphere in LYI Entamoeba medium supplemented with 10% HIBS and Diamond's Vitamin solution	Report results	Polymorphic, adherent, vacuoles visible
Genotypic Analysis ²		
Sequencing of 18S ribosomal RNA (rRNA) gene (~ 1840 base pairs)	Consistent with E. invadens	Consistent with E. invadens
Sequencing of <i>PpyLuc</i> gene (~ 340 base pairs) ³	≥ 99% sequence identity to <i>PpyLuc</i> (GenBank: M15077.1)	99.7% sequence identity to <i>PpyLuc</i> (GenBank: M15077.1)
Functional Activity of Luciferase Gene ^{2,4}	Positive	Positive
Viable Cell Count by Hemacytometry ²	> 10 ⁶ cells per mL	1.2 × 10 ⁶ cells per mL
Viability (post-freeze) ¹ 2 days at 25°C in a microaerophilic atmosphere in LYI Entamoeba medium supplemented with 10% HIBS and Diamond's Vitamin solution	Growth	Growth
Sterility (21-day incubation) ¹		
Harpo's HTYE broth, 37°C and 26°C, aerobic⁵	No growth	No growth
Trypticase soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth
DMEM with 10% FBS, 37°C and 26°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth

¹Testing completed on vialed, post-freeze material

/Heather Couch/

Heather Couch 31 MAR 2022

Program Manager or designee, ATCC Federal Solutions

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

ATCC® is a trademark of the American Type Culture Collection.

You are authorized to use this product for research use only. It is not intended for human use.

BEI Resources www.beiresources.org E-mail: contact@beiresources.org
Tel: 800-359-7370

Fax: 703-365-2898

²Testing completed on bulk material prior to vialing and freezing

³Primer sequences and conditions for PCR are available upon request.

⁴Luciferase activity was determined using the Luciferase Assay System (Promega E1500). Parasites were lysed and incubated with luciferase assay reagent. Luciferase activity was measured using a luminometer with a bioluminescence emission spectra of approximately 560 nm.

⁵Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.